Direct Generation of Electricity from Renewable Biomass Using Rumen Microorganisms as Biocatalysts in a Microbial Fuel Cell

Hamid Rismani-Yazdi[1], Ann D. Christy[1], Olli H. Tuovinen[2], Burk A. Dehority[3]

[1]Department of Food, Agricultural and Biological Engineering, [2]Department of Microbiology, [3]Department of Animal Sciences

1 Abstract

In microbial fuel cells (MFCs) bacteria generate electricity by mediating the oxidation of organic compounds and transferring the resulting electrons to an anode electrode. The objectives of this study were: 1) to test the possibility of generating electricity with rumen microorganisms as biocatalysts and cellulose as the electron donor in two-compartment MFCs, and 2) to characterize the microbial composition and electrochemical activity of rumen microorganisms enriched in MFCs. Maximum power density of 55 mW/m² was produced and sustained for over two months with periodic addition of cellulose. Cellulose hydrolysis and electrode reduction were shown to support the electricity generation in MFCs. Denaturing gradient gel electrophoresis of PCR amplified 16S rRNA genes revealed that the microbial communities differed when different substrates were used in the MFCs. The anode-attached and the suspended consortia were shown to be different within the same MFC. Cloning and sequencing analysis of 16S rRNA genes indicated that the most predominant bacteria in the anode-attached consortia were related to *Clostridium* spp., while *Comamonas* spp. were abundant in the planktonic consortia. MFC tests and cyclic voltammetry results suggested that bacteria were electrochemically active and transfer electrons via soluble electron shuttles excreted to the medium and also through the attachment of membrane-anchored biomolecules to the anodic electrode. The results demonstrated for the first time that electricity can be generated from cellulose by exploiting rumen microorganisms as biocatalysts, but both technical and biological optimization is needed to maximize the power output.

2 Introduction

Cellulosic biomass is one of the most abundant renewable sources of energy on earth. Chemical and biological approaches have been developed for production of ethanol, H_2 and methane from cellulosic materials, but these approaches encounter technical and economical hurdles. An alternative strategy is direct conversion of cellulose to electrical energy in microbial fuel cells (MFCs). MFCs are bioelectrochemical reactors in which microorganisms mediate the direct conversion of chemical energy stored in organic matter into electrical energy1. For direct conversion of cellulose to electricity in an MFC, the ideal microorganism(s) must be able to hydrolyze cellulose anaerobically and be electrochemically active, utilizing an anode as an alternative electron acceptor while oxidizing metabolites of cellulose hydrolysis. The rumen microbiota contains both strict and facultative anaerobes, which effectively hydrolyze cellulose and conserve energy via anaerobic respiration or fermentation.

Hypothesis: It is possible to convert chemical energy stored in cellulosic biomass into electricity using rumen microorganisms as biocatalysts in MFCs.

Objectives: 1) to test the possibility of generating electricity with rumen microorganisms as biocatalysts and cellulose as the electron donor in two-compartment MFCs, and 2) to characterize the microbial composition and electrochemical activity of rumen microorganisms enriched in MFCs.

3 Methods

Two-compartment MFCs were used with graphite plate electrodes (84 cm²) and Ultrex as proton-exchange membrane. Anolyte was habitat (rumen) simulating medium plus 10 % inoculum (strained rumen fluid). Catholyte in the cathode compartment was ferricyanide solution.

Electrochemical measurements

Power density (W/m²) was calculated as, $P = I \times V/A$, where V is cell voltage (V) across1000 Ω resistance, I ($I = V/R$) is current (amps), and A is the surface area of the electrode (m²). Polarization curve was generated by varying the resistance between anode and cathode from 1 MΩ to 9.9Ω after open circuit stabilization.

Microbial diversity analysis

Community DNA from anode biofilm and suspended microorganisms was extracted using the RBB+C method[2]. Analysis of microbial community structure was performed by resolving amplified 16s rRNA gene fragments on denaturing gradient gels. Phylogenetic analysis was carried out by producing libraries of genes encoding for 16S rRNA followed by sequencing.

Electrochemical characterization

Cyclic voltammetry was used to determine the electrochemical activity of bacteria enriched in MFCs. Production of reversible voltammogram peak(s) was used as an indicator for the electrochemical activity of the employed microorganisms.

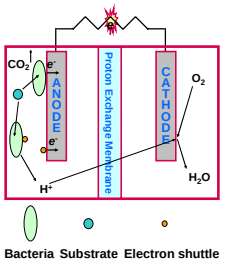


Figure 1. The working principle of a microbial fuel cell. Bacteria metabolize substrate is and transfer the resulting electrons to the anode.
This can occur either directly through the membrane or via electron shuttles.

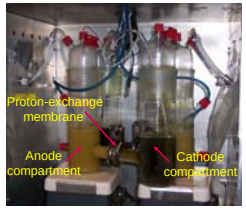


Figure 2. Experimental apparatus including replicate two-compartment MFCs within an incubation chamber.
Anode and cathode compartments are separated with an Ultrex proton-exchange membrane.

4 Results

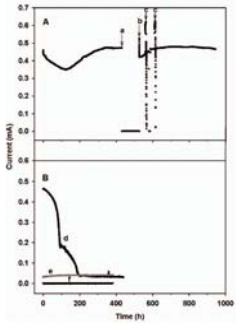


Figure 3. Electricity generation in two-compartment MFCs at 39±1°C. Voltage was monitored across 1000 ohm resistance between two 84 cm² graphite electrodes.
A. With rumen bacteria and cellulose. Manipulations: circuit opened (a), circuit closed (b), current fluctuation during polarization tests (c).
B. Controls with bacteria and without cellulose (d), without bacteria and with cellulose (e), without bacteria and with cellulose but without cysteine in the medium (f).

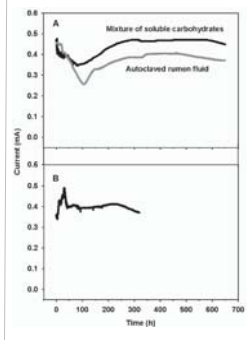


Figure 4. Electricity generation in MFCs at 39±1°C.
A. Rumen bacteria with a mixture of soluble carbohydrates and autoclaved rumen fluid as substrates.
B. Pre-enriched bacteria with cellulose as the substrate.

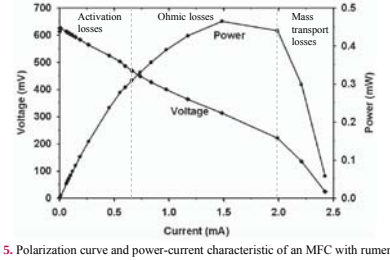
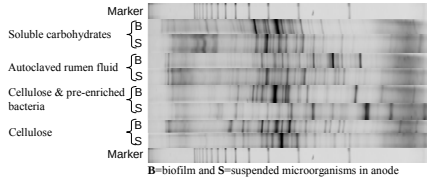


Figure 5. Polarization curve and power-current characteristic of an MFC with rumen bacteria as biocatalysts and cellulose as the electron donor at 39±1°C.
Maximum power density of 55.3 mW/m² with 211 Ω resistance.

Table 1. Closest matches with GeneBank sequences of cloned 16S rDNA gene fragment derived from anode biofilm (bacteria attached to the anode electrode) in cellulose-MFC.

Phylotype	Prevalence %	Closest BLAST match	% identity	RDP[1] classifier assignment[1]	Confidence (%)	Phylum
AA-78	1.1	*Clostridium*-like species	99	*Firmicutes*	61	*Firmicutes*
AA-12	8.9		94	*Clostridiaceae*	72	
AA-47	1.1		93	*Clostridiales*	75	
AA-75	1.1	Unidentified *Clostridiacea*	91	*Clostridiaceae*	94	
AA-24	1.1	Uncultured *Clostridium* sp.	91	*Butyrivibrio*	63	
AA-103	1.1	*Clostridium* sp.	94	*Clostridiaceae*	62	
AA-65	1.1		93	*Clostridiales*	87	
AA-100	6.7	*Clostridium*-like species	97	*Clostridiaceae*	70	
AA-94	1.1	*Clostridium straminisolvens*	95	*Acetivibrio*	98	
AA-22	1.1	*Clostridium thermocellum*	91	*Acetivibrio*	63	
AA-79	1.1	*Clostridium papyrosolvens*	90	*Acetivibrio*	92	
AA-8	1.1	*Clostridium aldrichii*	97	*Acetivibrio*	100	
AA-45	2.2	*Moorella thermoacetica*	82	*Sporanaerobacter*	70	
AA-33	1.1	*Acetivibrio cellulolyticus*	91	*Acetivibrio*	98	
AA-27	1.1	*Sedimentibacter* sp.	93	*Sedimentibacter*	99	
AA-11	5.6	*Sedimentibacter hongkongensis*	99	*Sedimentibacter*	73	
AA-55	2.2		98	*Clostridiales*	100	
AA-67	1.1		97	*Bacteria*	100	
AA-107	1.1	*Sedimentibacter hongkongensis*	94	*Soehngenia*	73	
AA-108	1.1	*Desulfotomaculum sp.*	99	*Clostridiales*	100	
AA-77	1.1		93	*Clostridiales*	83	
AA-41	2.2		89	*Clostridiales*	79	
AA-92	1.1		88	*Clostridiales*	77	
AA-6	1.1	*Desulfitobacterium hafniense*	89	*Desulfitobacterium*	100	
AA-98	1.1	*Desulfosporosinus* sp.	91	*Clostridiales*	67	
AA-48	1.1	*Ruminococcus* sp.	89	*Clostridiaceae*	95	
AA-46	2.2	*Ruminococcus flavefaciens*	86	*Clostridiaceae*	73	
AA-50	1.1	*Alicyclobacillus acidoterrestris*	82	*Clostridia*	65	
AA-39	2.2	*Bacillus* sp.	91	*Clostridiales*	71	
AA-102	1.1	Unidentified rumen bacterium	92	*Clostridiales*	94	
AA-83	1.1	Rumen bacterium R-7	85	*Clostridia*	75	
AA-10	6.7	*Geovibrio agilis*	95	*Geovibrio*	100	*Deferri-bacteres*
AA-34	2.2		88	*Geovibrio*	94	
AA-104	16.7	*Geovibrio ferrireductans*	98	*Geovibrio*	100	
AA-91	1.1		93	*Geovibrio*	98	
AA-16	1.1	*Clostridium straminisolvens*	85	*Geovibrio*	66	
AA-84	1.1	*Desulfovibrio desulfuricans*	98	*Desulfovibrio*	71	*Proteo-bacteria*
AA-1	2.2	*Comamonas* sp. 23310	99	*Comamonas*	100	
AA-18	1.1	*Pseudoxanthomonas mexicana*	99	*Pseudoxanthomonas*	78	
AA-90	1.1	*Pseudomonas boreopolis*	91	*Pseudoxanthomonas*	87	
AA-7	1.1	Uncultured *Pseudomonas* sp.	91	*Pseudomonadales*	60	
AA-80	1.1	*Rhizobium* sp.	98	*Bacteria*	91	
AA-15	1.1	*Ruminofilibacter xylanolyticum*	99	*Bacteroidales*	89	*Bacteroi-des*
AA-71	1.1	*Treponema* sp. Sy24	95	*Treponema*	99	*Spiro-chaetes*
AA-73	1.1		93	*Treponema*	74	
AA-38	3.3	*Treponema bryantii*	90	*Treponema*	98	

1. Ribosomal database project



B=biofilm and **S**=suspended microorganisms in anode

Figure 6. DDGE profiles of community DNA extracted from microbes that received four different substrates in MFCs.

Potential of a platinum working electrode was swept from -900 mV to 900 mV vs. an Ag/AgCl reference electrode in a 5-ml electrochemical cell with a scan rate of 100 mV/s.

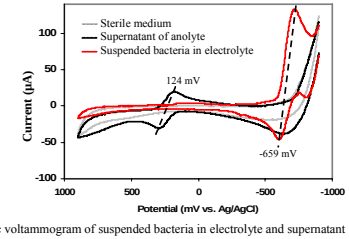


Figure 7. Cyclic voltammogram of suspended bacteria in electrolyte and supernatant of anolyte from cellulose-based MFCs with rumen bacteria as biocatalysts.

Table 2. Closest matches with GeneBank sequences of cloned 16S rDNA gene fragments derived from suspended bacteria in the anode compartment of cellulose-MFC

Phylotype	Prevalence %	Closest BLAST match	% identity	RDP[1] classifier assignment[1]	Confidence %	Phylum
SB-36	1.1	*Bacillales bacterium*	83	*Firmicutes*	88	*Firmicutes*
SB-78	1.1	*Bacillus* sp.	91	*Firmicutes*	100	
SB-21	1.1	*Butyrivibrio fibrisolvens*	83	*Clostridiales*	72	
SB-2	1.1	*Clostridium*-like species	97	*Clostridiaceae*	71	
SB-45	1.1		93	*Clostridiaceae*	77	
SB-34	1.1		89	*Clostridia*	96	
SB-14	1.1	*Desulfosporosinus orientis*	90	*Bacteria*	100	
SB-84	1.1	*Desulfotomaculum putei*	82	*Bacteria*	100	
SB-19	1.1	Bacterium	99	*Dethiosulfovibrio*	70	
SB-9	1.1	*Moorella glycerini*	89	*Clostridiales*	66	
SB-11	1.1		85	*Sporanaerobacter*	67	
SB-91	1.1	*Moorella thermoacetica*	83	*Sporanaerobacter*	68	
SB-100	37.5	*Comamonas* sp. 23310	99	*Comamonas*	100	*Beta-proteo-bacteria*
SB-10	1.1		98	*Comamonas*	100	
SB-101	1.1		96	*Comamonas*	100	
SB-28	2.3		95	*Comamonas*	100	
SB-30	1.1		88	*Comamonadaceae*	69	
SB-71	31	*Comamonas* sp. R-25060	96	*Comamonas*	99	
SB-82	1.1		91	*Comamonas*	90	
SB-37	2.3	*Pseudomonas boreopolis*	96	*Stenotrophomonas*	71	*Gamma-proteo-bacteria*
SB-68	1.1	*Pseudoxanthomonas mexicana*	98	*Pseudoxanthomonas*	82	
SB-93	2.3	*Pseudomonas* sp. An18	91	*Burkholderiales*	69	
SB-40	3.5	Unidentified rumen bacterium	99	*Rikenellaceae*	66	*Bacteroi-des*
SB-65	1.1	Unidentified rumen bacterium	95	*Tannerella*	82	

1. Ribosomal database project

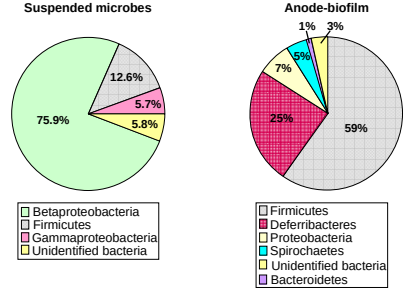


Figure 7. Phylogenic diversity of microbes sampled from cellulose-MFC

5 Conclusions

This study demonstrates for the first time that electricity can be generated from cellulose by exploiting rumen microorganisms as biocatalysts. rumen microbes are capable of hydrolyzing cellulose to metabolites that are respired with concomitant transfer of electrons to the anode. Electricity generation involves both anode-attached and suspended electrochemically active microorganisms with different community composition that changes with the substrate. The study also adds to the diversity of microorganisms that have been shown to produce electricity in MFCs. This system has potential for generating electricity from a wide range of agricultural and industrial cellulosic wastes such as crop residues and scrap paper, readily available in many parts of the world.

6 Acknowledgment

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7 References

1- Rabaey, K. and Verstraete, W. (2005) Trends Biotechnol. 23: 291-298.
2- Yu, Z. and Morrison, M. (2004) Biotechniques 36: 808-812.

8 Further information

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